Neuron

Supplemental Information

Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson's disease.

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SUPPLEMENTAL FIGURES

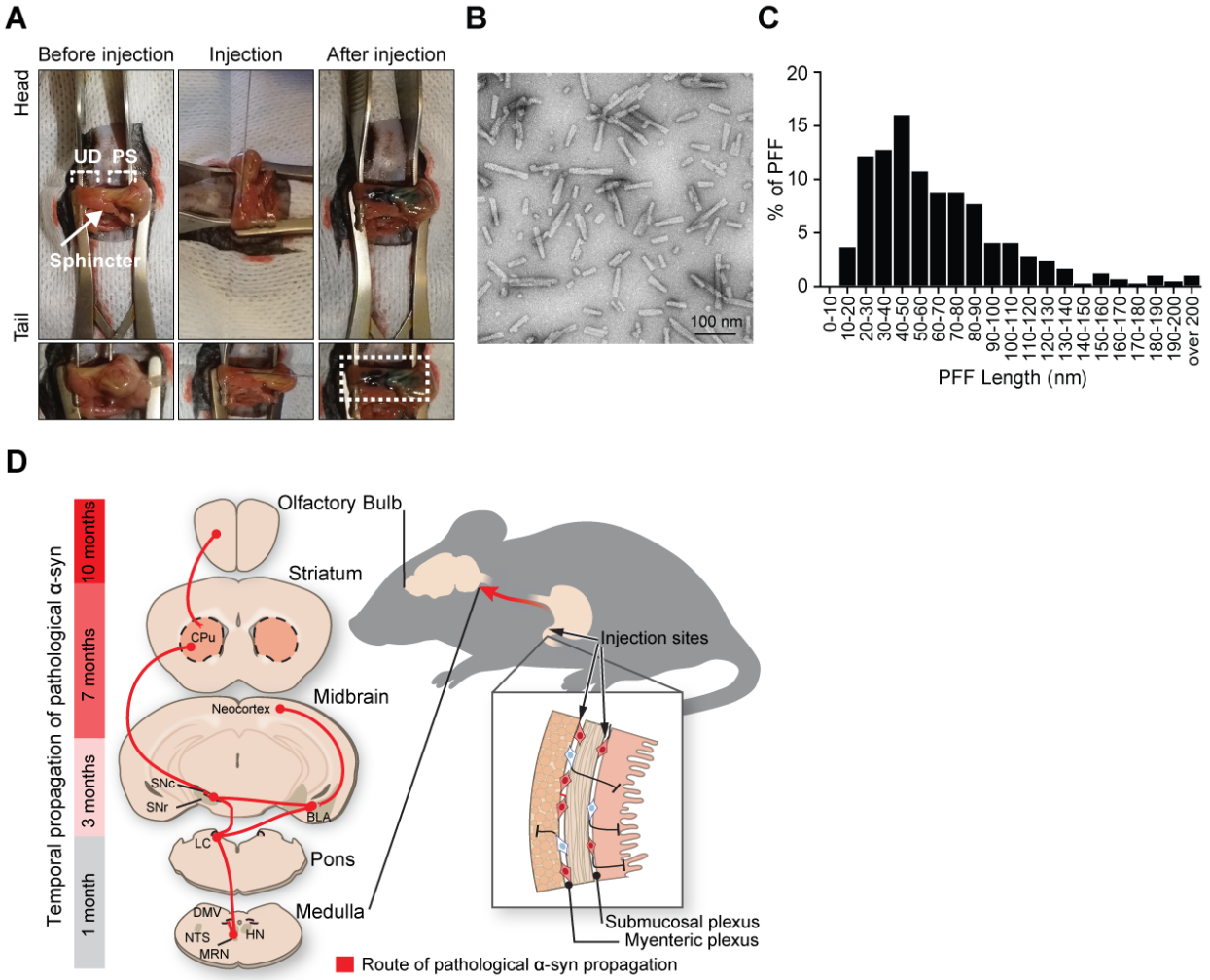


Figure S1. Confirmation of injection sites and the average size of α-Syn PFF, , Related to Figure 1.

(A) Confirmation of injection sites by trypan blue in the upper duodenum (UD) and pyloric stomach (PS).
(B) Electron microscopic image of mouse PFF. Scale bar represents 100 nm.
(C) Distribution of mouse α-Syn PFF length after sonication. Mean length of mouse PFF is 64.7 nm ± 1.7 nm (n=494).
(D) Diagram of temporal propagation of pSer129-α-syn.

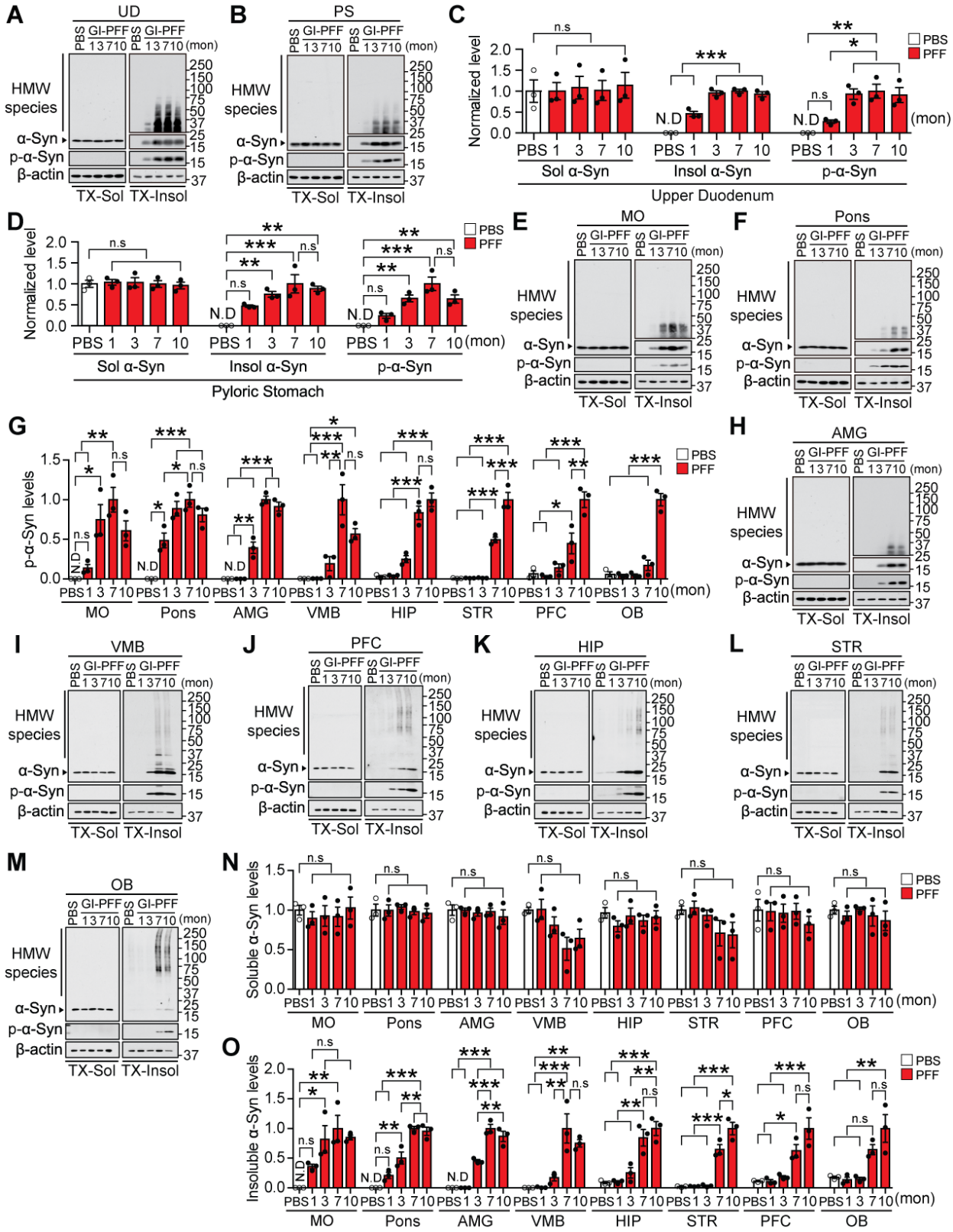


Figure S2. Characterization of transmission of pathological pS129-α-syn induced by α-syn PFF injection from the gut to the brain, Related to Figure 1.

(A and B) Representative immunoblots of α-syn, pSer129-α-syn and β-actin from the detergent (Triton x-100, TX) soluble (TX-Sol) and insoluble (TX-Insol) fraction of (A) the upper duodenum (UD) and (B) pyloric stomach (PS) from PBS injected mice or α-syn PFF UD and PS injected mice at 1, 3, 7 and 10 months.
(C and D) Quantifications of TX-Sol and TX-Insol α-syn and pS129-α-syn in (C) UD and (D) PS normalized to β-actin (n=3).
(E and F) Representative immunoblots of α-syn, pSer129-α-syn and β-actin from the TX-Sol and TX-Insol fraction from PBS injected mice or α-syn PFF UD and PS injected mice at 1, 3, 7 and 10 months. (E) medulla oblongata (MO) and (F) Pons.
(G) Quantification of TX-Insol pSer129-α-syn from MO to olfactory bulb (OB) normalized to β-actin (n=3).
(H-M) Representative immunoblots of α-syn, pSer129-α-syn and β-actin from the TX-Sol and TX-Insol fraction from PBS injected mice or α-syn PFF UD and PS injected mice at 1, 3, 7 and 10 months. (H) amygdala (AMG), (I) ventral midbrain (VMB), (J) pre-frontal cortex (PFC), (K) hippocampus (HIP), (L) striatum (STR), , (M) OB.
(N) Quantification of TX-Sol α-syn from MO to OB normalized to β-actin (n=3).
(O) Quantification of TX-Insol α-syn from MO to OB normalized to β-actin (n=3). Error bars represent the mean ± S.E.M. Statistical significance was determined using one-way ANOVA followed by post-hoc Bonferroni test for multiple group comparison. *P< 0.05, **P < 0.01, ***P < 0.001. n.s: not significant. N.D: not detected.

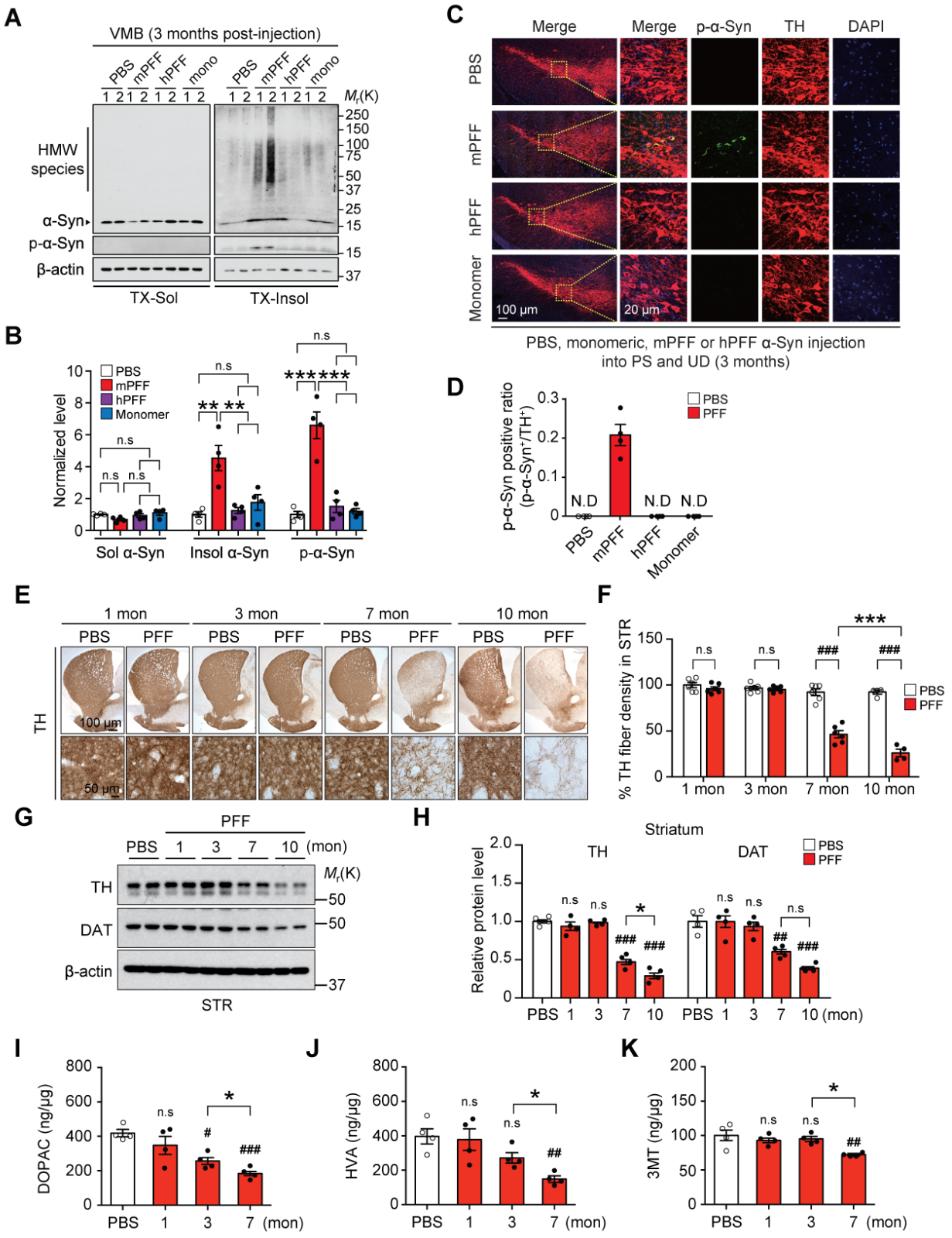


Figure S3. Comparison of mouse monomeric α-Syn PFF, mouse α-Syn PFF and human α-Syn PFF, and loss of dopaminergic terminal and dopamine in α-Syn PFF injection into the gut, related to Figures 1 and 2.

(A) Representative immunoblots of α-syn, pSer129-α-syn and β-actin from the detergent (Triton x-100, TX) soluble (TX-Sol) and insoluble (TX-Insol) fraction of the ventral midbrain (VMB) from PBS, mouse α-syn PFF (mPFF), human α-syn PFF (hPFF) or mouse α-syn monomer (mono) upper duodenum (UD) and pyloric stomach (PS) injected mice at 3 months.
(B) Quantification of TX-Sol and TX-Insol α-syn and pS129-α-syn normalized to β-actin (n=4).
(C) Representative double-immunostaining for pSer129-α-syn (Green) and TH (red) in the VMB 3 months after injection of PBS, mPFF, hPFF or mouse α-syn monomer.
(D) Quantification of pSer129-α-syn positive neurons normalized to TH positive neurons in the VMB (n=4).
(E) Representative photomicrograph of striatal sections stained for TH immunoreactivity 1, 3, 7 and 10 months after UD and PS injection of PBS or α-syn PFF. High power view of TH fiber density in the STR.
(F) Quantification of dopaminergic fiber densities in the STR (n=4-6) using Image J software (NIH).
(G) Time course of representative immunoblots of TH, DAT, and β-actin in the STR.
(H) Quantification of TH, and DAT protein levels normalized to β-actin (n=4).
(I–K) Striatal DA metabolite levels were measured by HPLC-ECD. Level of (I) DOPAC, (J) HVA, and, (K) 3MT were measured in the STR from PBS and α-syn PFF gastrointestinal injected mice (n=4). Error bars represent the mean ± S.E.M. Statistical significance was determined using one and two-way ANOVA followed by post-hoc Bonferroni test for multiple group comparison. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. PBS gastrointestinal injected group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. α-syn PFF gastrointestinal injected group. N.D: not detected. n.s: not significant.

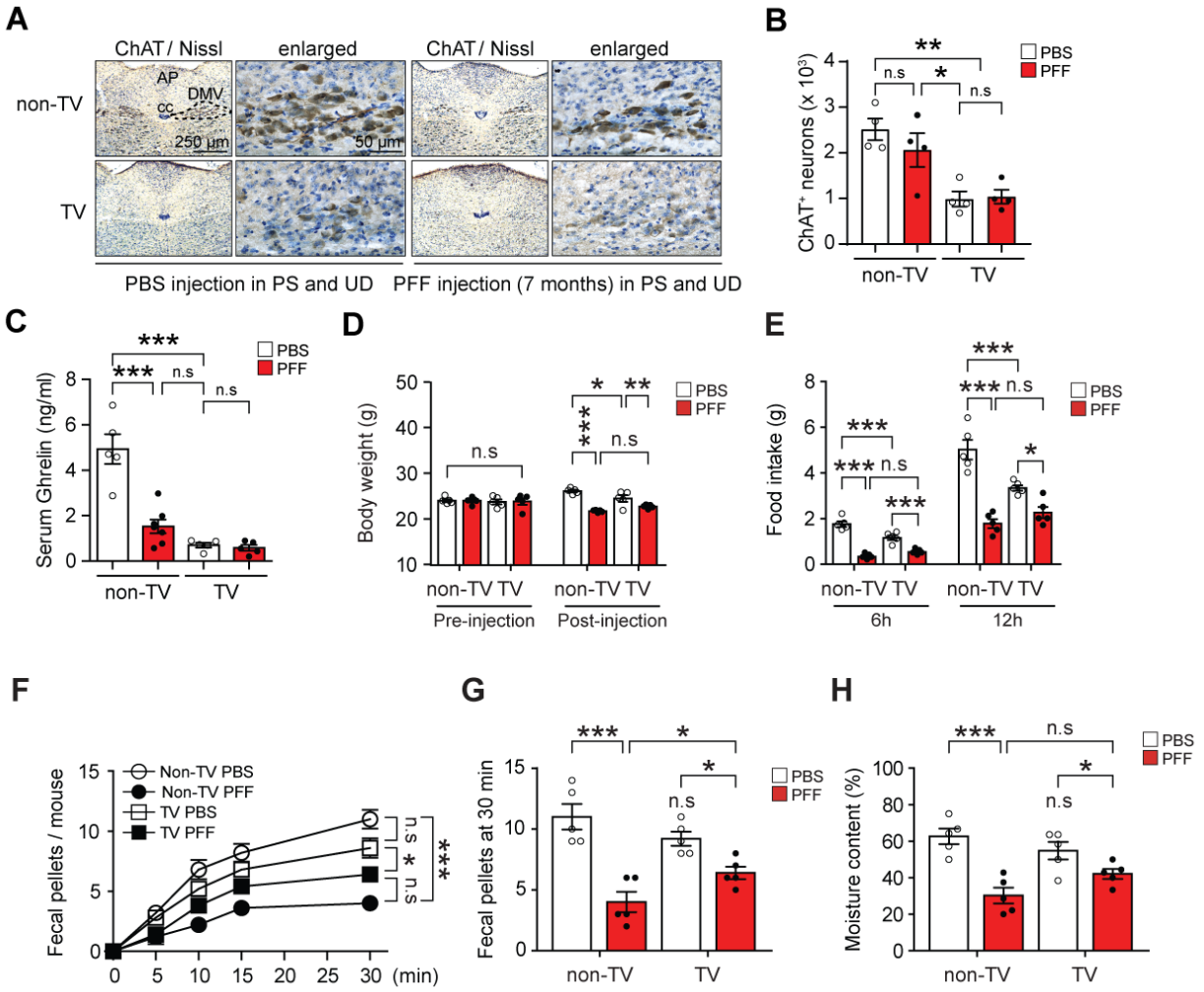


Figure S4. Confirmation of vagotomy and assessment of gastrointestinal function, Related to Figure 3.

(A) Representative photomicrographs of medulla oblongata sections stained for choline acetyl tranferase (ChAT) immunoreactivity from upper duodenum (UD) and pyloric stomach (PS) injected with PBS or α-syn PFF in mice that received a truncal vagotomy (TV) 7 months after the injection and mice that have an intact vagal nerve (non-TV).

(B) Unbiased stereology counts of ChAT-positive neurons in the dorsal motor nucleus of vagus (DMV) region of one hemisphere (n=4).

(C) Serum ghrelin levels (ng/ml) after 48 h of food deprivation from PBS or α-syn PFF UD and PS injected non-TV and TV mice 3 months after the injection.

(D) Body weight (gram, g) before and 1 month after injection of PBS or α-syn PFF in the UD and PS.

(E) 6 and 12 h food intake in PBS or α-syn PFF UD and PS injected non-TV and TV mice 1 month post-injection.

(F) Time course of fecal output in a novel environment over 30 min in PBS or α-syn PFF UD and PS injected non-TV and TV mice 1 month after injection.

(G) Total fecal pellets excreted in 30 min in PBS or α-syn PFF UD and PS injected non-TV and TV mice 1 month after injection. (H) The amount of water in the fecal pellet from

PBS or α-syn PFF UD and PS injected non-TV and TV mice 1 month post-injection. Error bars represent the mean ± S.E.M. Statistical significance was determined using a two-way ANOVA followed by post-hoc Bonferroni test for multiple group comparison. **P*< 0.05, ***P* < 0.01, ****P* < 0.001. n.s: not significant.

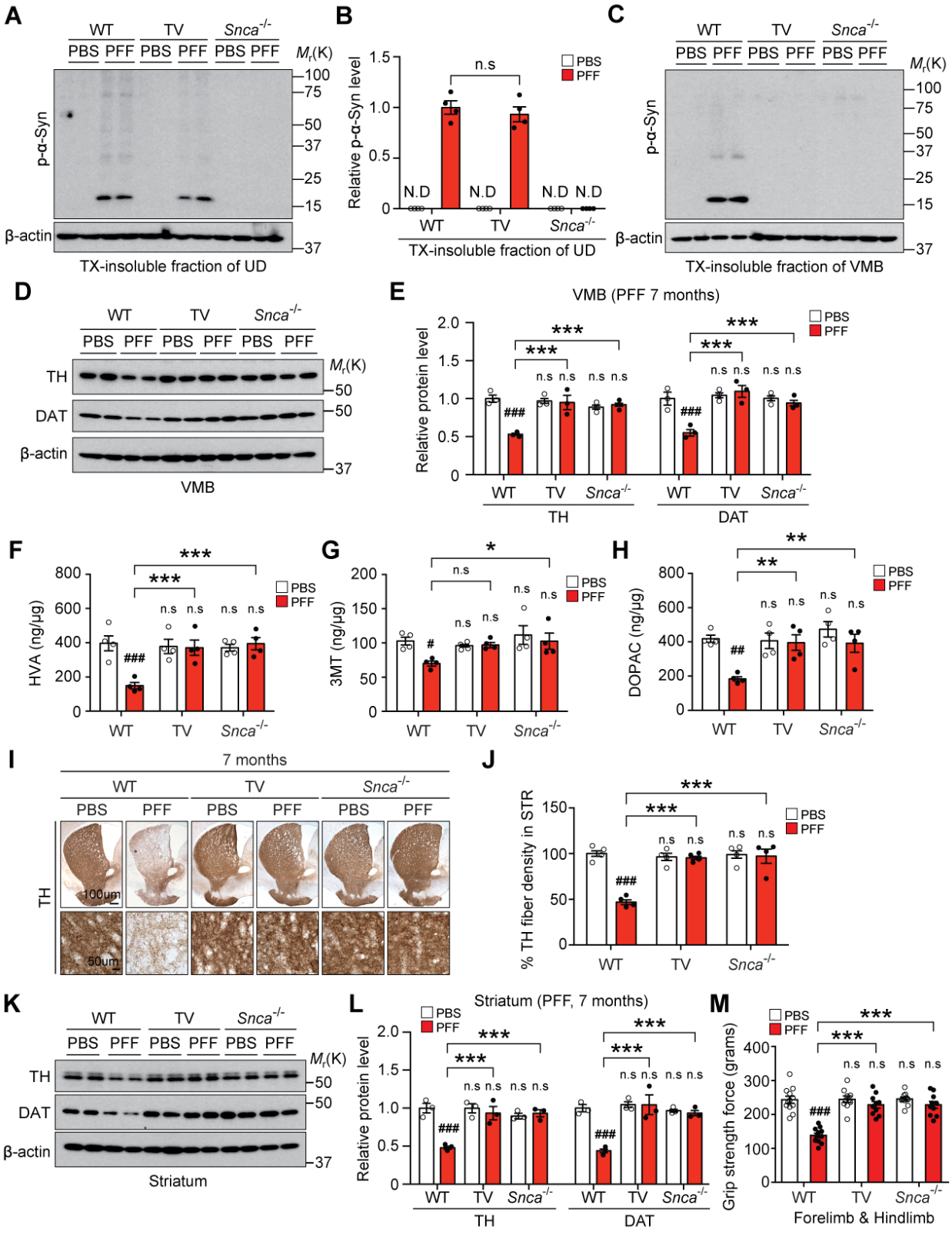
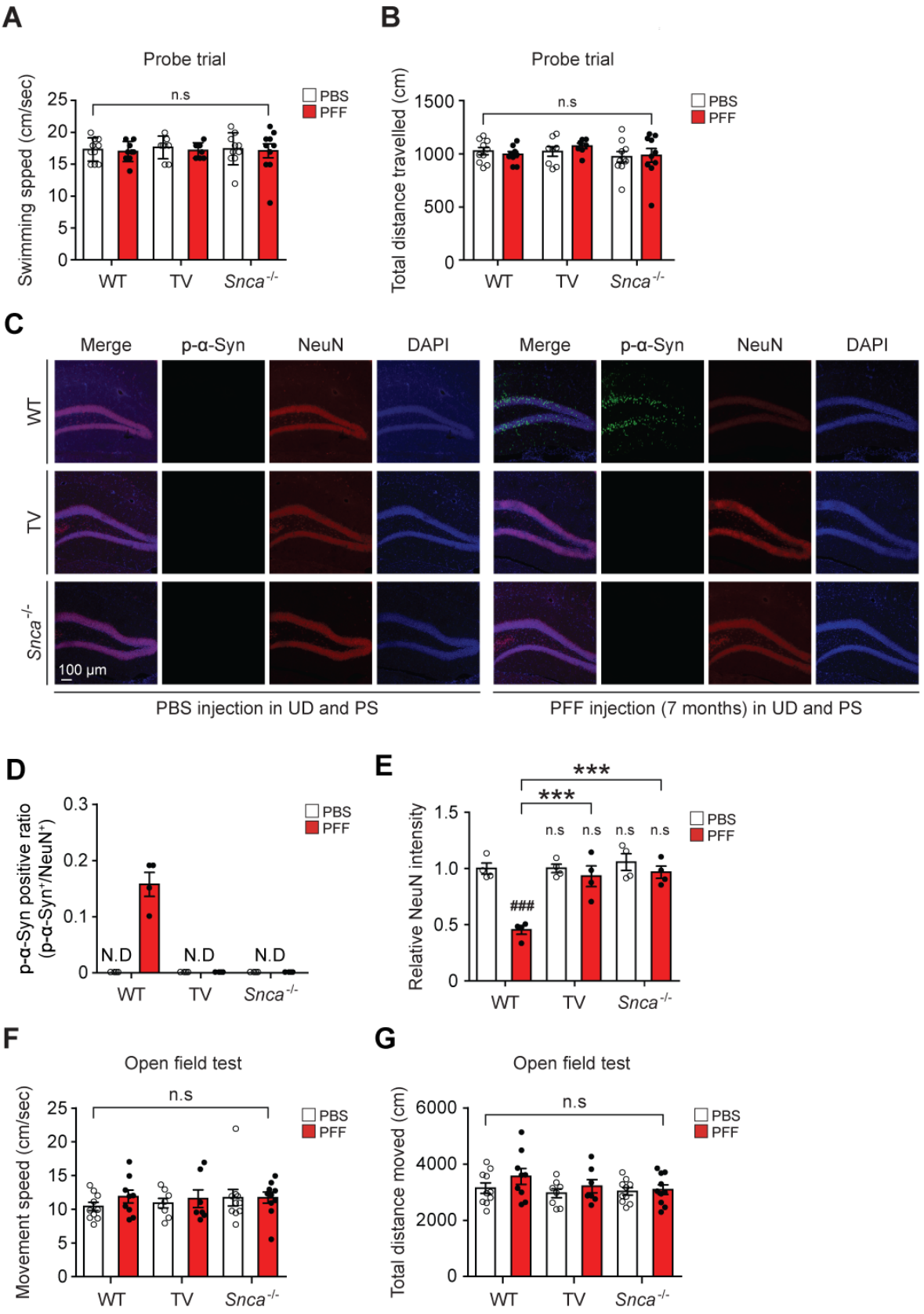


Figure S5. Vagotomy and α-syn deficiency prevent the accumulation and spread of pathological α-syn, dopaminergic terminal loss, dopamine depletion, and rescue progressive PD-like pathology induced by α-Syn PFF injection into the

gut, Related to Figure 3.
(A) Representative immunoblots of pSer129-α-syn and β-actin in the detergent (Triton x-100) insoluble fraction of the upper duodenum (UD) from PBS or α-syn PFF UD and pyloric stomach (PS) injected wild type (WT), vagotomy (TV) and *Snca*$^{-/-}$ mice 7 months after the injection.
(B) Quantification of pSer129-α-syn levels normalized to β-actin (n=4).
(C) Representative immunoblots of pSer129-α-syn and β-actin in the detergent (Triton x-100) insoluble fraction of ventral midbrain (VMB) from PBS or α-syn PFF UD and PS injected WT, TV and *Snca*$^{-/-}$ mice.
(D) Representative immunoblots of TH, DAT, and β-actin in VMB 7 months after α-syn PFF injection in the UD and PS in WT, TV and *Snca*$^{-/-}$ mice.
(E) Quantification of TH, and DAT protein levels normalized to β-actin (n=3). Striatal DA metabolites levels were measured by HPLC-ECD.
(F-H) Level of (F) HVA, (G) 3MT, and (H) DOPAC were measured in the STR from PBS and α-syn PFF gastrointestinal injected WT, TV and *Snca*$^{-/-}$ mice (n=4).
(I) Representative photomicrographs of striatal sections stained for TH immunoreactivity from α-syn PFF gastrointestinal injected WT, TV and *Snca*$^{-/-}$ mice 7 months after the injection. High power view of TH fiber density in the STR.
(J) Quantification of dopaminergic fiber densities in the STR (n=4) using Image J software (NIH).
(K) Representative immunoblots of TH, DAT, and β-actin in the STR fromα-syn PFF gastrointestinal injected WT, TV and *Snca*$^{-/-}$ mice 7 months after the injection.
(L) Quantification of TH, and DAT protein levels normalized to β-actin (n=3).
(M) Behavioral assessment of forelimb and hindlimb grip strength test of PBS and α-syn PFF gastrointestinal injected WT (n=11-12), TV (n=10) and *Snca*$^{-/-}$ mice (n=10) 7 months after the injection. Error bars represent the mean ± S.E.M. Statistical significance was determined using a two-way ANOVA followed by post-hoc Bonferroni test for multiple group comparison. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. PBS gastrointestinal injected WT group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. α-syn PFF gastrointestinal injected WT group. n.s: not significant. N.D: not detected.


A
Probe trial
Swimming spped (cm/sec)
n.s
PBS
PFF
WT
TV
Snca-/-
B
Probe trial
Total distance travelled (cm)
n.s
PBS
PFF
WT
TV
Snca-/-
C
Merge
p-α-Syn
NeuN
DAPI
WT
TV
Snca-/-
100 μm
PBS injection in UD and PS
PFF injection (7 months) in UD and PS
D
p-α-Syn positive ratio
(p-α-Syn+/NeuN+)
N.D
PBS
PFF
WT
TV
Snca-/-
E
Relative NeuN intensity
***
***
n.s
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PBS
PFF
WT
TV
Snca-/-
F
Open field test
Movement speed (cm/sec)
n.s
PBS
PFF
WT
TV
Snca-/-
G
Open field test
Total distance moved (cm)
n.s
PBS
PFF
WT
TV
Snca-/-

Figure S6. Vagotomy and α-syn deficiency do not affect speed or distance traveled following α-Syn PFF injection into the gut, Related to Figures 4 and 5.
Effect of vagotomy and α-syn deficiency on α-syn PFF-induced spatial memory defects in the Morris water maze trials (MWMT).
(A and B), (A) swimming speed and (B) total distanced travelled in probe trial sessions of the MWMT. Probe trial sessions were performed for 60 sec.
(C) Representative double-immunostaining for pSer129-α-syn (green) and NeuN (red) in the hippocampus 7 months post-injection of PBS or α-syn PFF in wild type (WT), truncal vagotomy (TV) and *Snca*$^{-/-}$ mice.
(D) Quantification of pSer129-α-syn immunoreactivity in the hippocampus (n=4).
(E) Quantification of NeuN intensity normalized to DAPI in the hippocampus (n=4).
(F and G) Effect of vagotomy and α-syn deficiency on α-syn PFF-induced locomotion and central activity in the open field test (OFT). The data of (F) total distance moved and (G) movement speed in the OFT. Mice were placed into the center of an open field and allowed to explore for 5 min under dim light. Error bars represent the mean ± S.E.M. Statistical significance was determined using a two-way ANOVA followed by post-hoc Bonferroni test for multiple group comparison. $^{###}P < 0.001$ vs. PBS UD and PS injected WT group. $^{***}P < 0.001$ vs. α-syn PFF UD and PS injected WT group. n.s: not significant.

SUPPLEMENTAL TABLE

Table S1. Comparison between mouse gastrointestinal injection models, Related to Figures 1 and 2.

	Holmqvist et al., *Acta Neuropathol*, 2014	Manfredsson et al., *Neurobiol Dis*, 2018	Uemura et al., *Mol Neurodegener*, 2018	This study
Injection site	Intestine wall of stomach and duodenum	Descending colon	Intestine wall of stomach	Intestine wall of stomach and duodenum
α-Syn species	PD lysates, Monomer, Oligomer, Fibril	PFF (<100 nm), Monomer	PFF (>200 nm)	PFF (<100 nm)
Dose	30 μg of PD lysates, 15 μg of Monomer, 15 μg of Oligomer, 15 μg of Fibril in Rat (250 g)	60 μg of Monomer, 60 μg of PFF in Rat (220 g)	48 μg of PFF in mouse (25 g)	25 μg of PFF in mouse (25 g)
Duration after injection	6 days	12 months	12 months	10 months
α-Syn propagation	Up to DMV	Up to DMV and LC	Up to DMV	DMV, LC, BLA, SN, HIP, STR, PFC, OB
Neurodegeneration in SN	No	No	No	Yes

Abbreviation: BLA, later to the basolateral amygdala; DMV, dorsal motor nucleus of the vagus; HIP, hippocampus; LC, locus coeruleus; OB, olfactory bulb; PFC, prefrontal cortex; PFF, Pre-formed fibrils; SN, substantia nigra; STR, striatum.

SUPPLEMENTAL MOVIES

Movie S1. Pole test, Related to Figure 3.
Movie S2. Morris water maze test, Related to Figure 4.
Movie S3. Elevated plus maze test, Related to Figure 5.
Movie S4. Open field test, Related to Figure 5.
Movie S5. Forced swimming test, Related to Figure 5.